Histamine receptor 2 is required to suppress innate immune responses to bacterial ligands in patients with inflammatory bowel disease

Smolinska, Sylwia ; Groeger, David ; Perez, Noelia Rodriguez ; Schiavi, Elisa ; Ferstl, Ruth ; Frei, Remo ; Konieczna, Patrycja ; Akdis, Cezmi A ; Jutel, Marek ; O Mahony, Liam

Abstract: BACKGROUND: Histamine is a key immunoregulatory mediator in immediate-type hypersensitivity reactions and chronic inflammatory responses, in particular histamine suppresses proinflammatory responses to bacterial ligands, through histamine receptor 2 (H2R). The aim of this study was to investigate the effects of histamine and H2R on bacteria-induced inflammatory responses in patients with IBD. METHODS: Peripheral blood mononuclear cells (PBMCs) were obtained from patients with Crohn's disease, patients with ulcerative colitis, and healthy controls. PBMC histamine receptor expression was evaluated by flow cytometry. Cytokine secretion following Toll-like receptor (TLR)-2, TLR-4, TLR-5, or TLR-9 stimulation in the presence or absence of histamine or famotidine (H2R antagonist) was quantified. Biopsy histamine receptor gene expression was evaluated using reverse transcription-polymerase chain reaction. The in vivo role of H2R was evaluated in the T-cell transfer murine colitis model. RESULTS: The percentage of circulating H2R monocytes was significantly reduced in patients with IBD. Histamine effectively suppressed TLR-induced cytokine secretion from healthy volunteer PBMCs but not for PBMCs from patients with IBD. Famotidine reversed this suppressive effect. H1R, H2R, and H4R gene expression was increased in inflamed gastrointestinal mucosa compared with noninflamed mucosa from the same patient and expression levels correlated with proinflammatory cytokine gene expression. Mice receiving lymphocytes from H2R donors, or treated with famotidine, displayed more severe weight loss, higher disease scores and increased numbers of mucosal IFN- and IL-17 T cells. CONCLUSION: Patients with IBD display dysregulated expression of histamine receptors, with diminished anti-inflammatory effects associated with H2R signaling. Deliberate manipulation of H2R signaling may suppress excessive TLR responses to bacteria within the gut.

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Histamine Receptor 2 is Required to Suppress Innate Immune Responses to Bacterial Ligands in Patients with Inflammatory Bowel Disease

Sylwia Smolinska, PhD,[,†] David Groeger, PhD,[‡,§] Noelia Rodriguez Perez, PhD,[‡] Elisa Schiavi, PhD,[‡] Ruth Ferstl, PhD,[‡] Remo Frei, PhD,[‡,¶] Patrycja Konieczna, PhD,[‡] Cezmi A. Akdis, MD,[‡] Marek Jutel, MD, PhD,[*,†] and Liam O'Mahony, PhD[‡]*

Background: Histamine is a key immunoregulatory mediator in immediate-type hypersensitivity reactions and chronic inflammatory responses, in particular histamine suppresses proinflammatory responses to bacterial ligands, through histamine receptor 2 (H_2R). The aim of this study was to investigate the effects of histamine and H_2R on bacteria-induced inflammatory responses in patients with IBD.

Methods: Peripheral blood mononuclear cells (PBMCs) were obtained from patients with Crohn's disease, patients with ulcerative colitis, and healthy controls. PBMC histamine receptor expression was evaluated by flow cytometry. Cytokine secretion following Toll-like receptor (TLR)-2, TLR-4, TLR-5, or TLR-9 stimulation in the presence or absence of histamine or famotidine (H_2R antagonist) was quantified. Biopsy histamine receptor gene expression was evaluated using reverse transcription–polymerase chain reaction. The in vivo role of H_2R was evaluated in the T-cell transfer murine colitis model.

Results: The percentage of circulating H_2R^+ monocytes was significantly reduced in patients with IBD. Histamine effectively suppressed TLR-induced cytokine secretion from healthy volunteer PBMCs but not for PBMCs from patients with IBD. Famotidine reversed this suppressive effect. *H_1R, H_2R,* and *H_4R* gene expression was increased in inflamed gastrointestinal mucosa compared with noninflamed mucosa from the same patient and expression levels correlated with proinflammatory cytokine gene expression. Mice receiving lymphocytes from $H_2R^{-/-}$ donors, or treated with famotidine, displayed more severe weight loss, higher disease scores and increased numbers of mucosal IFN-γ^+ and IL-17^+ T cells.

Conclusion: Patients with IBD display dysregulated expression of histamine receptors, with diminished anti-inflammatory effects associated with H_2R signaling. Deliberate manipulation of H_2R signaling may suppress excessive TLR responses to bacteria within the gut.

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Key Words: histamine, IBD, TLR

Crohn's disease and ulcerative colitis are the 2 major forms of inflammatory bowel disease (IBD), and both diseases are chronic immune-mediated disorders of the gastrointestinal tract, associated with significant morbidity and health care costs. The precise mechanisms driving these diseases remain unknown but complex interactions between the immune system, enteric microbiota, and host genotype underlie the development of IBD.[1] Components of the intestinal microbiota can drive protective or pathological responses in IBD models, highlighting the importance of host–microbe communication in the development of these diseases.[2,3] The innate immune system responds to microbial components or products through activation of pattern recognition receptors, including Toll-like receptors (TLRs), which are transmembrane pattern recognition receptors located either on the cell surface or in the endosomes.[4] Several regulatory mechanisms that control TLR activation have been described, thus preventing excessive inflammatory responses to the resident microbiota.[5] In particular, many host-derived and environment metabolites can influence immune reactivity and TLR activation, with significant effects on mucosal immune homeostasis.[6] Coordination between

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From the *Department of Clinical Immunology, Wroclaw Medical University, Wroclaw, Poland; †"ALL-MED" Medical Research Institute, Wroclaw, Poland; ‡Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland; §Alimentary Health Pharma Davos, Davos, Switzerland; and ¶Christine Kühne-Center for Allergy Research and Education, Zurich, Switzerland.

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Reprints: Liam O'Mahony, PhD, Swiss Institute of Allergy and Asthma Research, Obere Strasse 22, 7270 Davos Platz, Switzerland (e-mail: liam.omahony@siaf.uzh.ch).

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multiple pattern recognition receptor signaling pathways is important for the induction of the appropriate innate and adaptive response. For example, TLR-2 recognition of a *Bifidobacterium* strain promotes dendritic cell interleukin (IL)-10 and retinoic acid secretion, whereas tumor necrosis factor alpha (TNF-α) and IL-12 secretion is suppressed.[7]

Histamine is a biogenic amine, which induces a range of immunoregulatory activities in innate and adaptive immune cells.[8] Four histamine receptors have been described (H_1R–H_4R), and histamine-induced immune effects are dependent on the type of receptor expressed by a specific cell.[9] H_2R has been shown to exert regulatory effects in multiple models. Dendritic cell proinflammatory responses to microbial ligands are significantly suppressed by means of histamine binding to the H_2R, whereas suppression of IL-27 secretion was mediated by means of both H_2R and H_4R.[10,11] Murine H_2R knock out mice display defects in gastric and immune regulatory functions, whereas mucosal expression of H_2R is critical for immunomodulatory responses to bacteria-derived histamine.[12,13] T-cell expression of H_2R has dramatic effects on lymphocyte polarization, whereas H_2R expression by allergen-specific T_H2 cells directly suppresses allergen-stimulated T cells but promotes IL-10 production.[14,15] Interestingly, mucosal histamine levels, but not plasma levels, were shown to be increased in patients with IBD, and increased urinary levels of N-methylhistamine, a stable histamine metabolite, correlated with disease activity in both patients with Crohn's disease and patients with ulcerative colitis.[16–18] However, expression of the different histamine receptors and the functional effects of histamine signaling on TLR responses to microbial ligands have not been previously described for patients with IBD. We describe that histamine does not suppress proinflammatory cytokine responses to TLR ligands as effectively for peripheral blood mononuclear cells (PBMCs) from patients with IBD when compared with healthy controls, which correlates with reduced H_2R expression by peripheral blood monocytes. Histamine receptor expression is increased in inflamed mucosa, whereas blocking H_2R in a murine T-cell transfer colitis model exacerbates disease.

MATERIALS AND METHODS

Human Studies

In this study, 22 healthy volunteers, 21 patients with Crohn's disease and 21 ulcerative colitis patients were recruited from the Department of Clinical Gastroenterology of IV Military Hospital and First Department and Clinic of General, Gastroenterological and Endocrinological Surgery in Wroclaw, Poland. Patient demographic details are included in Table 1. All patients and healthy volunteers read and signed an informed consent form before recruitment. The study-specific procedures were approved by the Wroclaw Medical University Ethics Committee. The diagnosis of Crohn's disease or ulcerative colitis was based on clinical, radiological, and endoscopic examination and histological findings. Patients were excluded if immunosuppressive medications were taken within 1 month of blood sampling. All recruited patients were in the active stage of disease. Following recruitment, 50 mL of peripheral blood was obtained from each volunteer. Biopsy-derived complementary DNA from 10 patients with Crohn's disease and 10 ulcerative colitis patients were provided by the Swiss IBD Cohort Biobank (http://ibdcohort.ch). Written informed consent was obtained before specimen collection, and studies were approved by the regional Swiss Ethics Committees in which cohort participants were enrolled.

TABLE 1. Patient Demographics

	Healthy Controls	Crohn's Disease	Ulcerative Colitis	*P*
n	22	21	21	
Age (SD)	37.9 (13.0)	38.7 (11.5)	43.0 (15.2)	0.241
Male/female	9/13	11/10	13/8	0.386
Disease duration, yr		4.9	4.4	0.697
Current therapy				
5-Aminosalicylates		13	16	0.317
Biologics		1	0	0.315
Nutritional therapy[a]		9	2	0.014
PPI		4	1	0.153
Antibiotics		7	1	0.018

[a]Nutritional therapy includes multivitamins, iron, and/or vitamin B supplementation.

PBMCs Isolation and Cryopreservation

Mononuclear cells were isolated from 50 mL of peripheral blood using the Boyüm method. After resuspending blood in phosphate-buffered solution, without calcium and magnesium, cells were separated by density gradient centrifugation on ficoll. The mononuclear cell layer was collected and washed 2 times in phosphate-buffered solution. Cells were counted using a Neubauer chamber and then resuspended in freezing medium (45% fetal calf serum [FCS], 45% Roswell Park Memorial Institute medium [RPMI], 10% dimethyl sulfoxide [DMSO]) at a concentration of 1×10^6 cells per milliliter. Cells were stored in liquid nitrogen until further analysis.

In Vitro Cell Culture

Following careful resuscitation and acclimatization of the PBMCs at 37°C, cells were cultured in cRPMI media (Gibco; Thermo Fisher Scientific, Waltham, MA) at a concentration of 1×10^6 cells per milliliter. Cells were stimulated with the TLR ligands Pam3Cys (0.5 μg/mL; Calbiochem, Darmstadt, Germany), lipopolysaccharide (LPS) (500 ng/mL; Sigma-Aldrich, Buchs, Switzerland), flagellin (100 ng/mL; Invivogen, San Diego, CA), or CpG (100 μM; Microsynth, Balgach, Switzerland) or remained unstimulated for 24 hours. In total, 1×10^{-5}M histamine (Sigma-Aldrich) was added 10 minutes before TLR ligands, whereas the H_2R antagonist famotidine (Sigma-Aldrich) was added 30 minutes before histamine at a concentration of 1×10^{-5}M. Supernatants were collected after 24 hours, and cytokines

were quantified using the Bioplex Multiplex Suspension Array System (Bio-Rad Laboratories, Hercules, CA).

Flow Cytometry Analysis

All flow cytometry analyses were performed on the Gallios Flow Cytometer (Beckman Coulter, Brea, CA). Antibodies to CXCR3, T-bet, IFN-γ (T_H1 panel), CCR4, CRTh2, GATA-3, IL-5, IL-13 (T_H2 panel), CD161, CCR6, ROR-γt, IL-17 (T_H17 panel), CD3, CD4, CD14, and CD19 were obtained from eBioscience (Vienna, Austria). Labeled anti-human H_1R antibody was obtained from R&D Systems (Minneapolis, MN). Anti-human H_2R (GeneTex; Lucerna-Chem, Luzern, Switzerland) and anti-human H_4R (Alomone Labs, Jerusalem, Israel) were labeled using DyLight Fluorescent Dyes (Thermo Scientific, Schwerte, Germany). Staining with antihistamine receptor antibodies was validated in cell lines and primary cells with known levels of histamine receptor gene expression. Each batch of antibodies was validated before use because significant batch-to-batch variations were noted. Only one validated batch of antibody was used for the entire study. Flow cytometry results were analyzed in Kaluza Flow Analysis Software (Beckman Coulter).

Gene Expression

PBMC messenger RNA was isolated using RNeasy Mini Kit and QIA-Shredder-Kit (Qiagen, Venlo, the Netherlands). Reverse transcription was performed using RevertAid RT Kit (Thermo Fisher Scientific). Real-time polymerase chain reaction was performed with iTaq SYBR Green Supermix with ROX (Bio-Rad Laboratories). The primers used are listed in Supplementary Table S1, Supplemental Digital Content 1, http://links.lww.com/IBD/B274, and all were synthesized by Microsynth.

Severe Combined Immunodeficient T-cell Transfer Colitis Model

The C.B-17 severe combined immunodeficient (SCID) mice were obtained from Charles River (Sulzfeld, Germany) and maintained under specific pathogen-free conditions. The animals were housed at the AO Research Institute (Davos, Switzerland) in individually ventilated cages for the duration of the study, and all experimental procedures were carried out in accordance with Swiss law. Colitogenic $CD4^+CD25^-CD45RB^{high}$ cells were isolated from BALB/c wild-type or BALB/c $H_2R^{-/-}$ donor mouse spleens using the MACS system (depletion of $CD4^+CD25^+$ cells followed by positive selection of CD45RB FITC-labeled cells). Colitis was induced by intraperitoneal transfer of 4×10^5 cells per C.B-17 SCID mouse at day 0. Half of the animals, which received wild-type T cells, were daily injected intraperitoneally with famotidine (20 μmol/kg), whereas the remaining animals received daily NaCl injections intraperitoneally. Disease severity scores were recorded, whereas animal weights were monitored every day. Disease severity scores included feces condition (1 = wet; 2 = diarrhea; 3 = bloody diarrhea or rectal prolapse), activity (1 = isolated, abnormal position; 2 = huddled, hypoactive, or hyperactive; 3 = unconscious), coordination of movement (1 = slightly uncoordinated; 2 = very uncoordinated; 3 = paralysis), and fur quality (1 = reduced grooming; 2 = disheveled; 3 = hair loss). Following euthanasia on day 22, mesenteric lymph nodes were isolated and single-cell suspensions generated using mechanical means. IFN-γ^+ and IL-17^+ lymphocytes were quantified by flow cytometry.

Statistical Analysis

Statistical significance between experimental conditions was calculated using the nonparametric *t* test Mann–Whitney, whereas correlations were assessed using the Pearson test. When more than 2 groups were compared, a 1-way analysis of variance and Dunnett post-hoc test were used to determine statistical significance. Paired *t* tests were used to assess the differences between gene expression in inflamed versus noninflamed mucosa. All data analysis was carried out using GraphPrism software (GraphPad Sofware Inc., La Jolla, CA).

RESULTS

Monocyte H_2R Expression is Downregulated in Patients with IBD

Histamine receptor expression was evaluated on peripheral blood mononuclear cells (PBMCs) using flow cytometry. H_1R expression by peripheral blood $CD14^+$ monocytes was not different between the groups. However, there was a decrease in H_2R^+ monocytes, associated with an increase in H_4R^+ monocytes, in the peripheral blood of patients with Crohn's disease and ulcerative colitis (Fig. 1A). Few $CD3^+$ T cells expressed H_1R or H_2R, with greater numbers expressing H_4R. No differences in T-cell histamine receptor expression were observed between the groups (Fig. 1B). The histamine receptor most frequently expressed by $CD19^+$ B cells was H_2R; however, there was no difference in expression between the groups. Very few B cells express H_1R or H_4R, but significantly more H_4R^+ B cells were observed in the peripheral blood of patients with ulcerative colitis (Fig. 1C). An example of the gating strategy is illustrated in Figure 1D.

In addition, we analyzed lymphocyte polarization in peripheral blood by flow cytometry. T_H1 cells were identified as IFN-γ^+, T-bet$^+$, and CXCR3$^+$ and T_H2 cells as CRTh2$^+$, GATA-3$^+$, CCR4$^+$, IL-5$^+$, and IL-13$^+$, whereas T_H17 cells were CD161$^+$, ROR-γt$^+$, CCR6$^+$, and IL-17$^+$. Both patients with Crohn's disease and ulcerative colitis had elevated T_H1 cells (Fig. 2A) and T_H17 cells (Fig. 2B) compared with healthy controls, with the increases in patients with Crohn's disease reaching statistical significance. However, no differences in T_H2 cells were observed between the groups (Fig. 2C). Interestingly, a significant inverse association was noted between the percentage of H_2R^+ monocytes and the percentage of T_H17 cells in peripheral blood (Fig. 2D). No significant correlation was observed between the percentage of H_2R^+ monocytes and the percentage of T_H1 or T_H2 cells in peripheral blood (Fig. 2E, F).

Cytokine Responses to TLR Ligands is Modulated by Histamine Through H_2R

PBMCs from healthy controls, patients with Crohn's disease, and patients with ulcerative colitis were stimulated in vitro

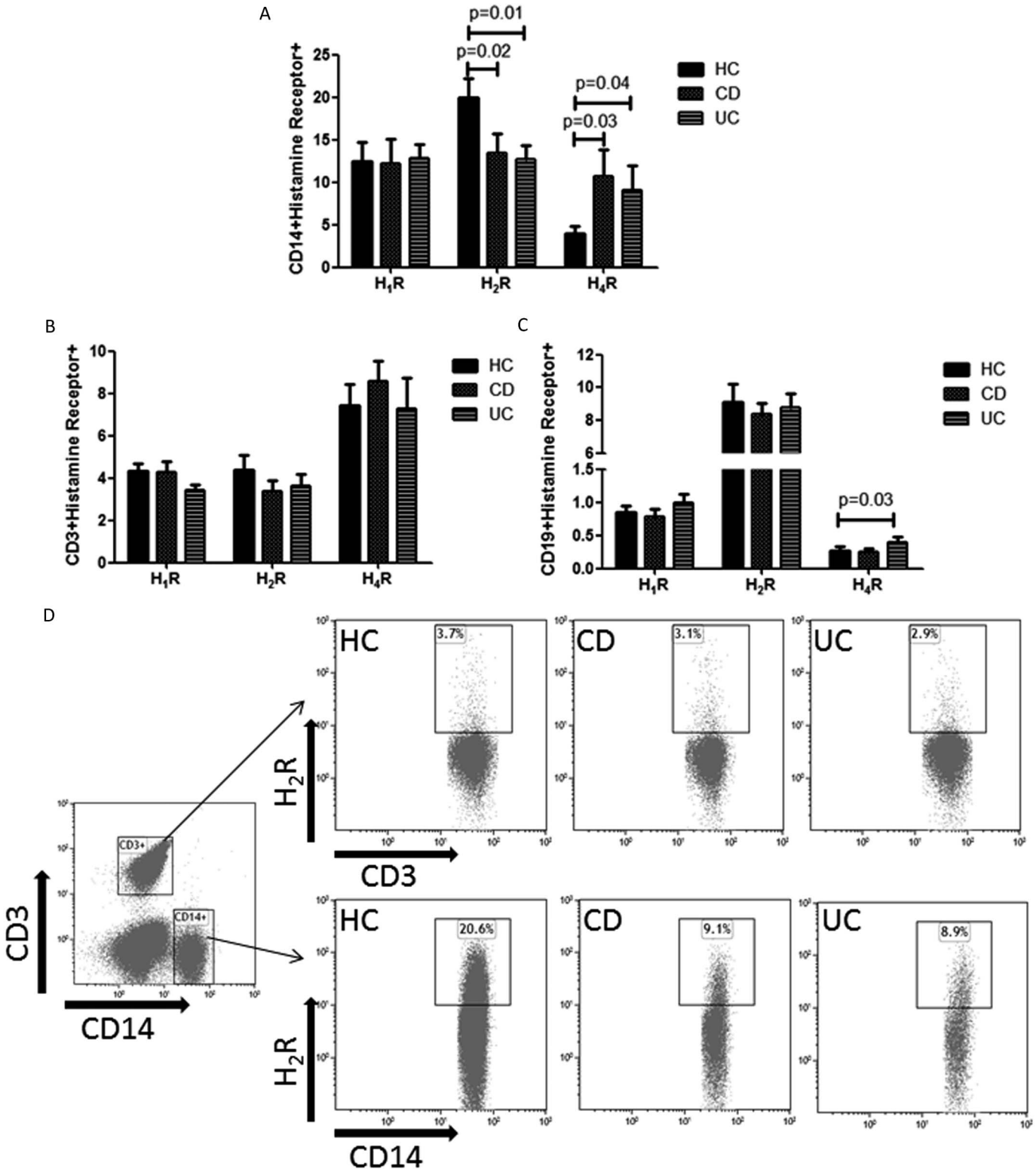

FIGURE 1. Low H_2R expression is accompanied by high H_4R expression on monocytes from patients with IBD. PBMCs were examined by flow cytometry for the expression of H_1R, H_2R, and H_4R. $CD14^+$ monocytes (A), $CD3^+$ T-cell (B), or $CD19^+$ B-cell (C) histamine receptor expression was quantified as a percentage of the population. A representative example of the gating strategy is illustrated (D).

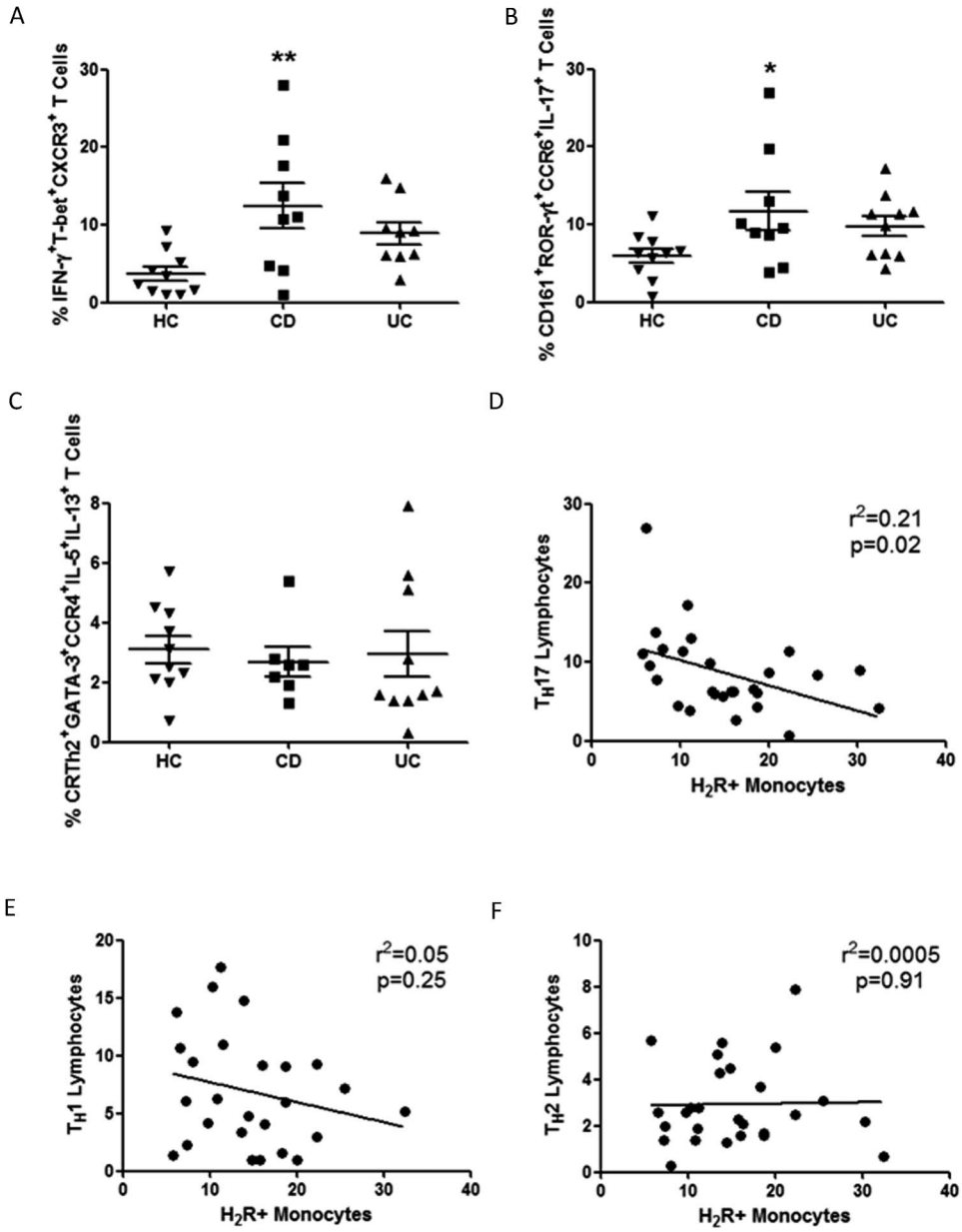

FIGURE 2. T_H1 and T_H17 lymphocytes are increased in patients with IBD. T_H1 lymphocytes (A) and T_H17 lymphocytes (B) were elevated in both patients with Crohn's disease (CD) and patients with ulcerative colitis (UC) compared with healthy controls (HC). No change in T_H2 cells (C) was observed between the groups. The percentage of T_H17 cells in peripheral blood was negatively correlated with the percentage of H_2R^+ monocytes in peripheral blood (D). No correlations were observed for the percentage of H_2R^+ monocytes in peripheral blood and the percentage of T_H1 cells (E) or T_H2 cells (F). *$P < 0.05$; **$P < 0.01$.

with the TLR ligands Pam3Cys (TLR-2), LPS (TLR-4), flagellin (TLR-5), or CpG oligonucleotides (TLR-9) in the presence or absence of histamine. Histamine significantly reduced TNF-α secretion, stimulated by all TLR ligands; however, significantly greater suppression of TNF-α section was observed for TLR-2, TLR-4, and TLR-5 stimulated PBMCs from healthy controls compared with PBMCs from patients with Crohn's disease or ulcerative colitis (Fig. 3A). Similarly, IL-6 secretion was suppressed to a significantly greater extent by histamine for TLR-2 stimulated PBMCs from healthy controls compared with patients with Crohn's disease and for TLR-5 stimulated PBMCs from healthy controls compared with patients with Crohn's disease or ulcerative colitis (Fig. 3B). Histamine also suppressed TLR-2 and TLR-5 stimulated IFN-γ secretion significantly more in PBMCs from healthy controls compared with both disease groups (Fig. 3C), whereas suppression of IL-12p70 secretion by histamine was significantly greater for TLR-2 or TLR-9 stimulated healthy control PBMCs (Fig. 3D). Histamine suppression of MCP-1 secretion

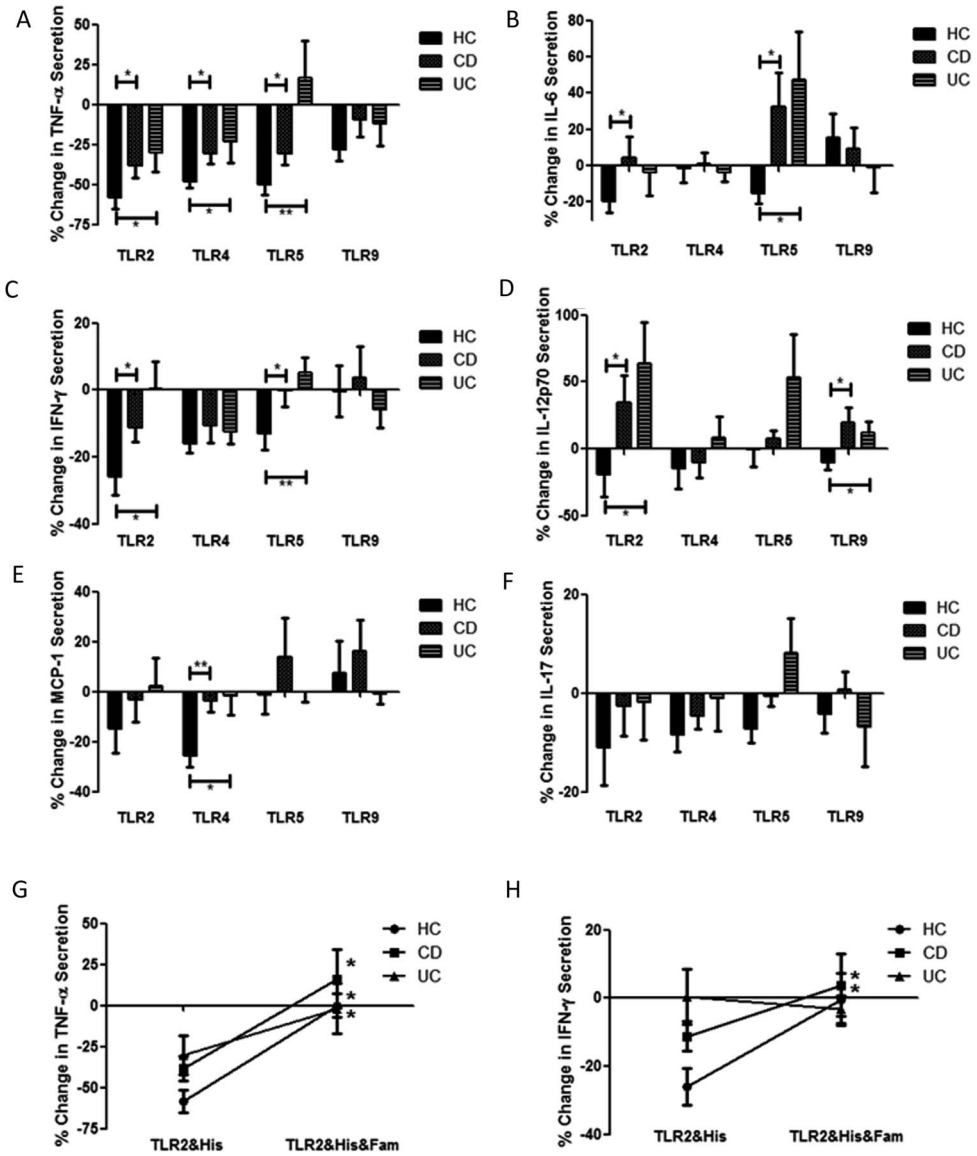

FIGURE 3. Histamine suppression of TLR-induced cytokine responses is significantly less for PBMCs from patients with IBD. Cytokine secretion from in vitro TLR-stimulated PBMCs was suppressed by histamine. Inhibition of TNF-α (A), IL-6 (B), IFN-γ (C), IL-12p70 (D), and MCP-1 (E) secretion was significantly greater for PBMCs co-incubated with histamine from healthy controls (HC) compared with patients with Crohn's disease (CD) or ulcerative colitis (UC). TLR-stimulated IL-17 secretion was not significantly suppressed (F). Inclusion of the H_2R antagonist famotidine completely reversed histamine suppression of TLR-2 stimulated TNF-α (G) and TLR-2 stimulated IFN-γ (H). Results are expressed as percentage change in cytokine secretion from TLR-stimulated compared with TLR- and histamine-stimulated PBMCs. *$P < 0.05$; **$P < 0.01$.

by TLR-4 activation was more effective for PBMCs from healthy controls (Fig. 3E). Finally, there was no significant difference between the groups for the influence of histamine on IL-17 secretion (Fig. 3F). The differential effects of histamine on TLR-induced cytokine secretion was not because of differences in cytokine levels between the groups following TLR-stimulation alone. Secretion of TNF-α, IL-6, IFN-γ, IL-12p70, MCP-1, and IL-17 in response to TLR-2, TLR-4, TLR-5, and TLR-9 stimulation was similar for PBMCs from healthy controls, patients with Crohn's disease, and patients with ulcerative colitis (see Fig. S1, A–F, Supplemental Digital Content 2, http://links.lww.com/IBD/B275).

Differences in TLR expression may be associated with altered responses to their ligands. Therefore, we quantified TLR expression in peripheral blood using reverse transcription–polymerase chain reaction. There was no difference in the expression of *TLR-2*, *TLR-4*, or *TLR-5* between the groups (see Fig. 2, A–C, Supplemental Digital Content 3, http://links.lww.com/IBD/B276). However, *TLR-9* expression was significantly reduced in the peripheral blood of ulcerative colitis patients compared with healthy controls (see Fig. 2D, Supplemental Digital Content 2, http://links.lww.com/IBD/B275). Expression of the genes related to histamine synthesis (*HDC*) and metabolism (*HNMT* and *DAO*) were also quantified in

peripheral blood (Fig. 4). Although *HDC* and *HNMT* gene expression was similar for all groups, *DAO* gene expression was lower in patients with Crohn's disease and statistically significantly lower for patients with ulcerative colitis compared with healthy volunteers (Fig. 4).

To determine if H_2R was responsible for the histamine effects on TLR-stimulated PBMCs, famotidine was used to block the H_2R. Famotidine reversed the inhibitory effect of histamine on TLR-2 stimulated secretion of TNF-α (Fig. 3G) and TLR-2 stimulated secretion of IFN-γ (Fig. 3H).

Histamine Receptors Are Upregulated in the Inflamed Mucosa

From each patient, mucosal biopsies were obtained from inflamed and noninflamed regions, as determined by endoscopy. Histamine receptor expression was quantified using reverse transcription–polymerase chain reaction. Inflammatory activity was confirmed by quantifying *IFN-γ* and *TNF-α* gene expression. Both *IFN-γ* and *TNF-α* gene expression was significantly upregulated in the biopsies from inflamed mucosa compared with noninflamed mucosa (Fig. 5A, B). H_1R expression was upregulated in inflamed mucosa from patients with ulcerative colitis but not in inflamed mucosa from patients with Crohn's disease (Fig. 5C). H_2R and H_4R gene expression were increased in inflamed mucosa from both patients with Crohn's disease and patients with ulcerative colitis (Fig. 5D, E). Within the inflamed mucosa of patients with ulcerative colitis, there was a significant positive correlation between H_4R gene expression and *IFN-γ* gene expression, whereas H_2R gene expression correlated with *TNF-α* gene expression (Fig. 5F, G). No correlations between histamine receptor gene expression and *TNF-α* or *IFN-γ* gene expression were observed for patients with Crohn's disease. Expression of the genes related to histamine synthesis (*HDC*) and metabolism (*HNMT* and *DAO*) were similar for inflamed and noninflamed mucosa (see Fig. 3, A–F, Supplemental Digital Content 4, http://links.lww.com/IBD/B277).

Loss of H_2R Exacerbates Colitis in a Murine Model

Using the T-cell transfer model of colitis,[19] we determined if H_2R influences disease development. SCID mice received either T_{REG}-depleted T cells from wild-type mice, T_{REG}-depleted T cells from H_2R-deficient mice, or T_{REG}-depleted T cells from wild-type mice, and in addition, these animals were treated daily with famotidine. Weight loss began earlier and was more pronounced in animals receiving T cells from H_2R-deficent mice or animals administered famotidine (Fig. 6A). Similarly, disease severity scores were elevated in these animals (Fig. 6B). Furthermore, significantly more IFN-γ^+ and IL-17^+ lymphocytes were observed in animals receiving T cells from H_2R-deficent mice or animals administered famotidine (Fig. 6C, D). Colonic myeloperoxidase levels were significantly elevated in mice receiving H_2R-deficient lymphocytes, with a trend for increased myeloperoxidase levels in mice treated with famotidine (Fig. 7A). Colonic *TNF-α* and *IL-17* gene expression was significantly increased in both mice treated with famotidine or H_2R-deficient lymphocytes, whereas IFN-γ gene expression was significantly increased in mice receiving H_2R-deficient lymphocytes with a nonstatistical significant increase for IFN-γ gene expression in mice treated with famotidine (Fig. 7B). H_1R, H_2R, and H_4R gene expression increased in the colon of colitic animals, with no differences in expression being observed for mice treated with famotidine or mice receiving H_2R-deficient lymphocytes compared with mice receiving wild-type lymphocytes (Fig. 7C).

DISCUSSION

Inflammatory bowel diseases are chronic debilitating inflammatory disorders that affect millions of people worldwide. Even though great progress has been made in understanding the basic inflammatory mechanisms that underpin mucosal inflammation, significant gaps have prevented the development of meaningful new therapies. Inappropriate and exaggerated immune responses to microbial factors within the gut are thought to play a key role in the pathogenesis of IBD and therefore a better understanding of the regulatory molecules, which dampen these responses, would be highly valued. In this report, we present evidence that histamine signaling is disrupted in both patients with Crohn's disease and patients with ulcerative colitis. Histamine receptor expression and functional activity are altered, which was previously unrecognized in these patients. In addition,

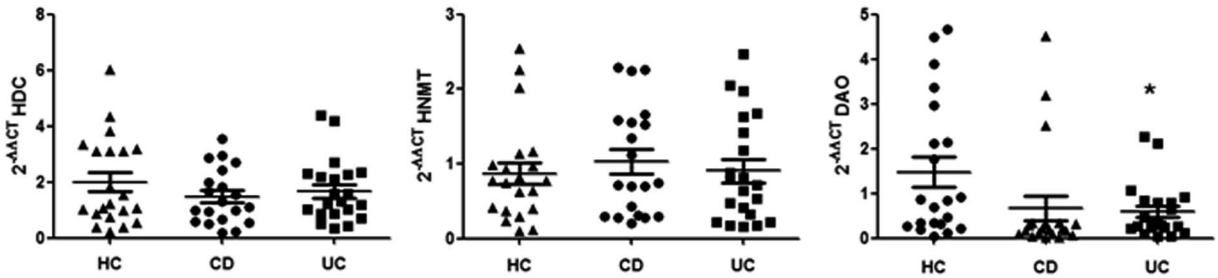

FIGURE 4. Decreased DAO gene expression in peripheral blood. Peripheral blood HDC and HNMT gene expression were similar for healthy controls (HC), patients with Crohn's disease (CD), and patients with ulcerative colitis (UC). However, DAO gene expression in peripheral blood was decreased for patients with CD and statistically significantly decreased for UC patients. *$P < 0.05$.

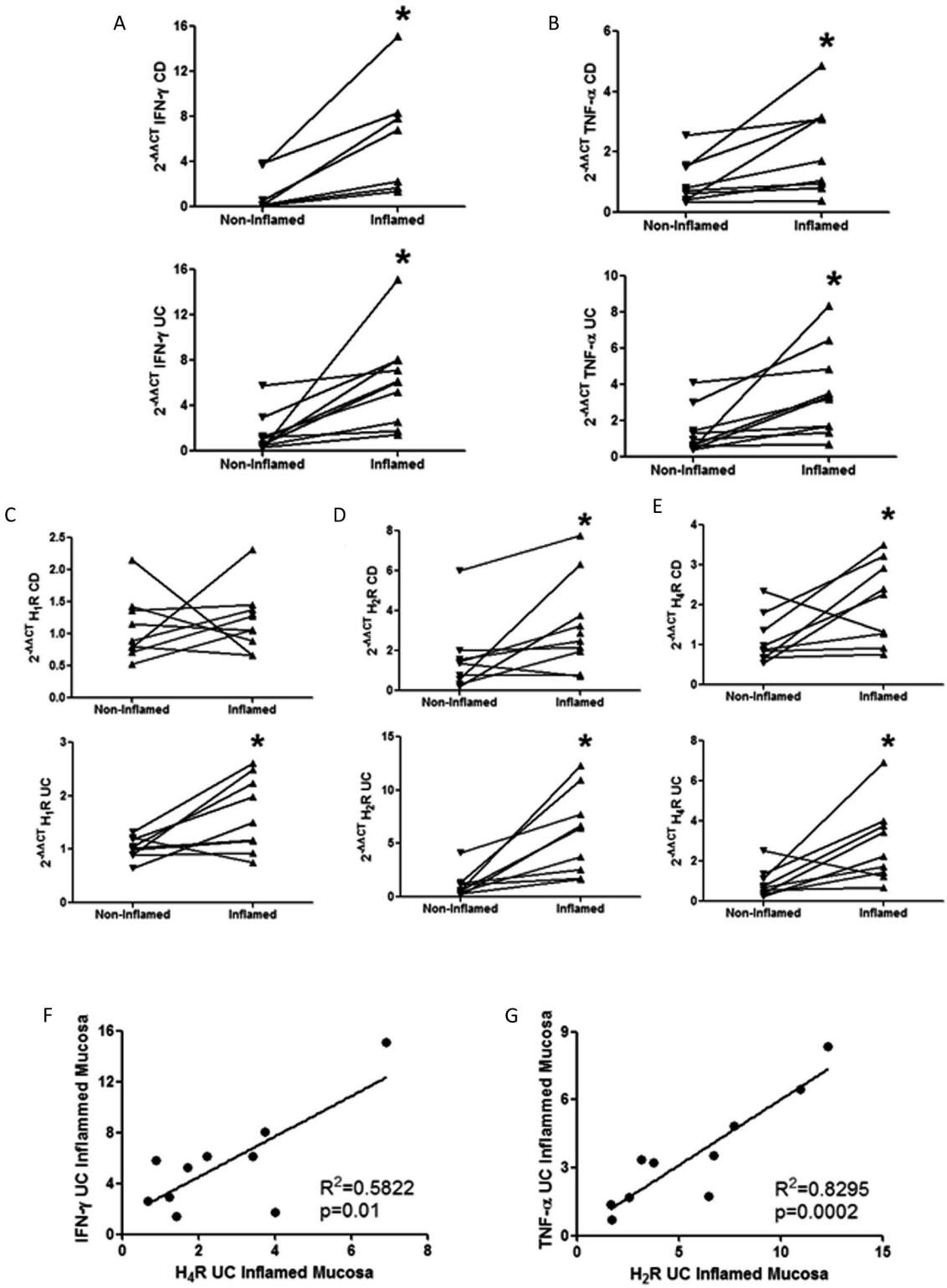

FIGURE 5. Increased expression of histamine receptor genes within the inflamed mucosa. Expression of IFN-γ (A) and TNF-α (B) was significantly increased in inflamed versus noninflamed biopsies from patients with Crohn's disease (CD) and ulcerative colitis (UC). H_1R (C) was elevated in inflamed mucosa from UC but not from patients with CD. H_2R (D) and H_4R (E) were both elevated in inflamed mucosa from CD and UC patients. IFN-γ gene expression correlates with H_4R expression (F), whereas TNF-α expression correlates with H_2R (G) in inflamed mucosa from UC patients. *$P < 0.05$.

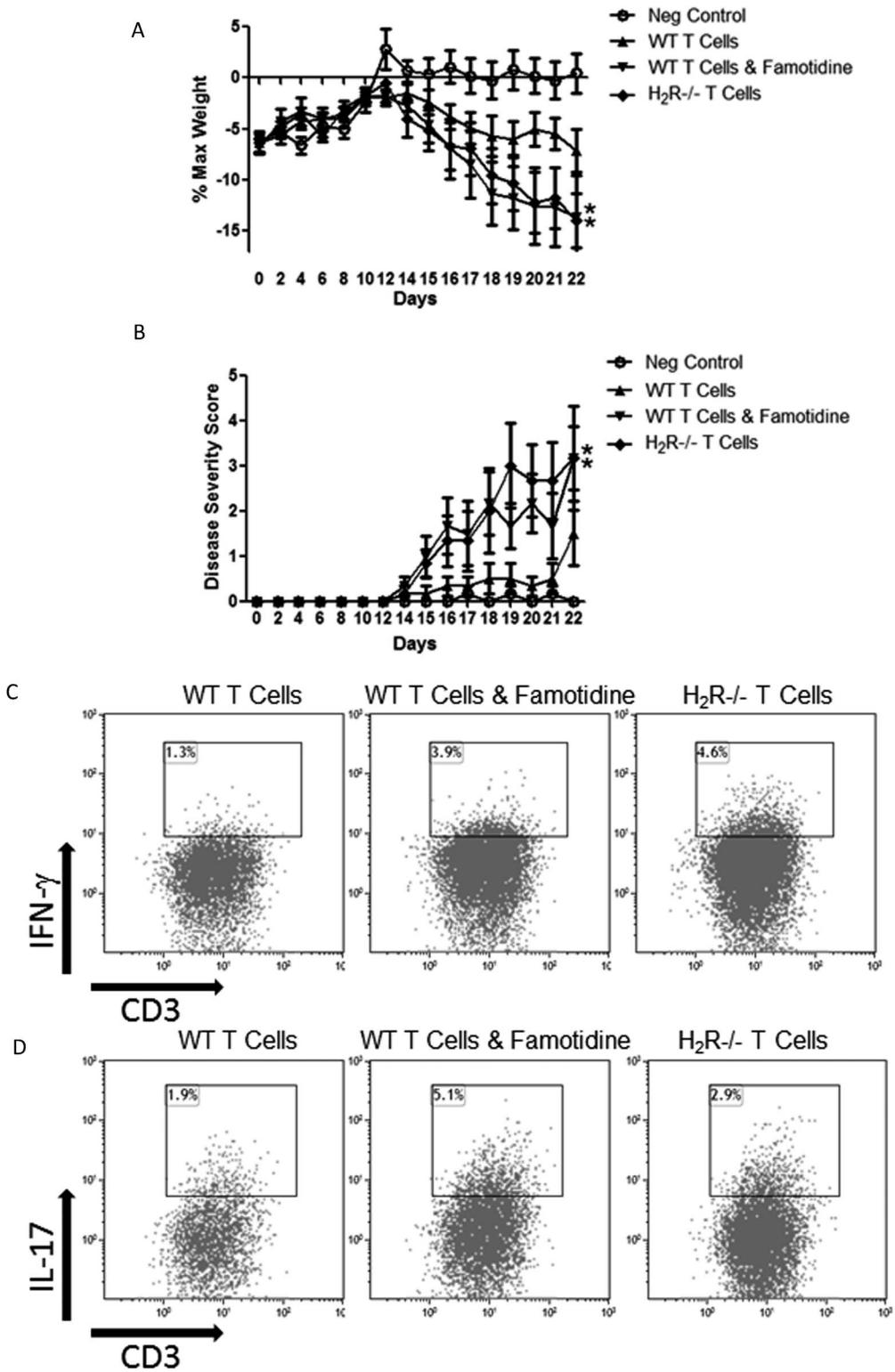

FIGURE 6. Loss of H_2R activity exacerbates disease in the T-cell transfer colitis model. Mice lost weight faster when lymphocytes were adoptively transferred from $H_2R^{-/-}$ animals or when transferred with wild-type (WT) lymphocytes and treated with famotidine during the study (A). Similarly, disease severity scores were elevated when H_2R signaling was blocked (B). IFN-γ^+ (c) and IL-17^+ (D) lymphocytes were increased in the mesenteric lymph nodes when H_2R was blocked. *$P < 0.05$.

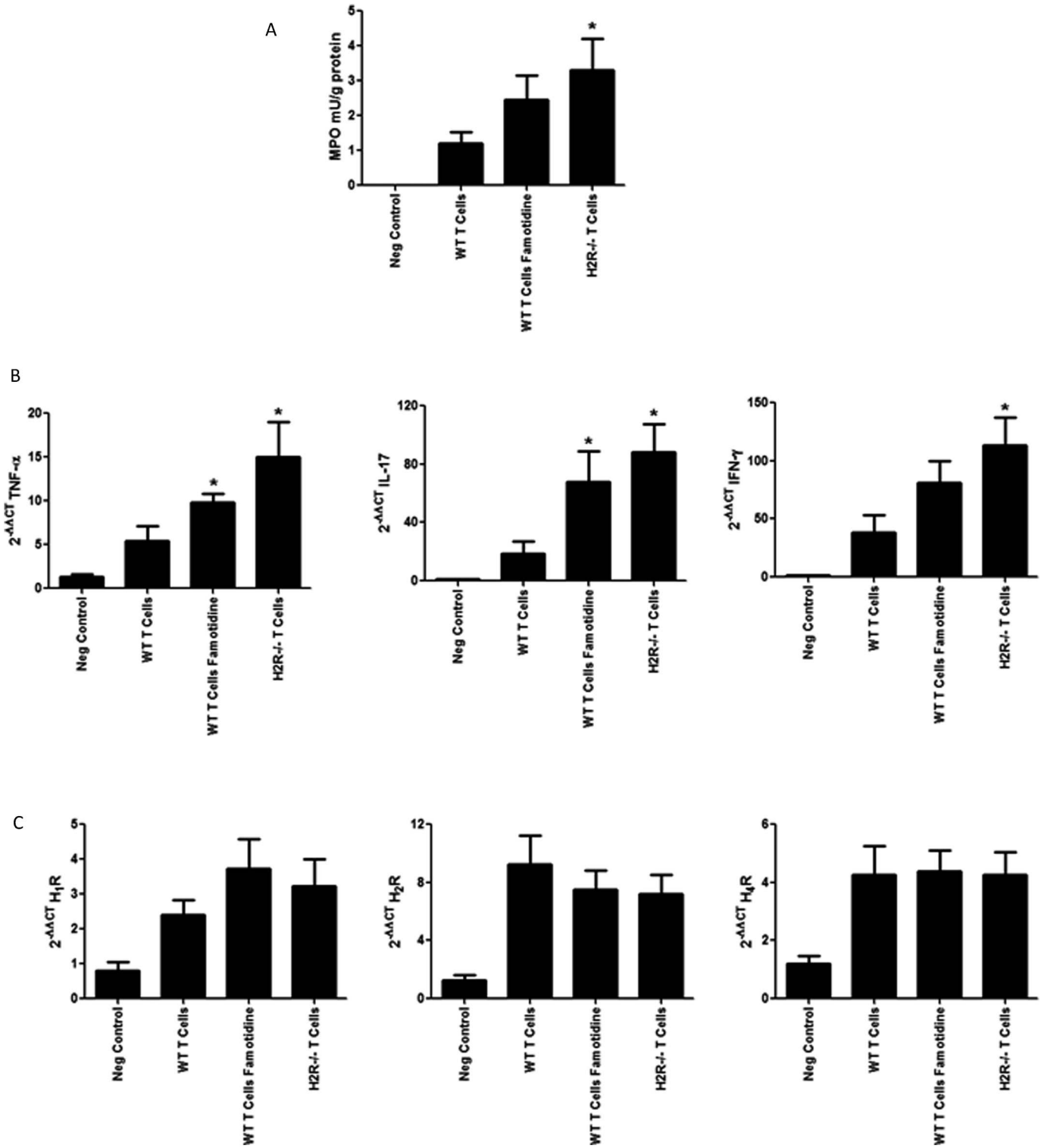

FIGURE 7. Loss of H_2R activity increases colonic myeloperoxidase (MPO) levels and cytokine gene expression. Mice receiving lymphocytes from $H_2R^{-/-}$ animals had significantly elevated MPO levels compared with mice receiving wild-type (WT) lymphocytes (A). Similarly, TNF-α, IL-17, and IFN-γ gene expression were elevated when H_2R signaling was blocked (B). H_1R, H_2R, and H_4R gene expression were elevated in mice following the induction of colitis (C). *$P < 0.05$.

blocking H_2R results in more severe inflammatory disease in the murine T-cell transfer colitis model.

Histamine has been previously shown to significantly influence the intensity and polarization of immune responses, including the recognition of microbes by TLRs.[10,20–22] Using a panel of TLR ligands, we demonstrate that histamine exerts a diminished suppressive effect on cells from patients with IBD, possibly due to the decreased number of H_2R^+ monocytes. The decrease in H_2R^+ monocytes is negatively correlated with T_H17 cells in peripheral blood, which parallels the animal model data where increased IL-17^+ lymphocytes were found in the mucosa of animals treated with a H_2R antagonist or animals receiving H_2R-deficient lymphocytes. However, histamine did not directly suppress TLR-induced IL-17 secretion from PBMCs, suggesting a direct influence of H_2R on T_H17 lymphocyte polarization, which may be independent of the effect of histamine on IL-17 secretion by innate immune cells, such as monocytes. The reasons for the monocytes switching from H_2R to H_4R expression are unknown but may include the influence of circulating proinflammatory cytokines. H_4R activation can promote T_H17 responses in murine models, and H_4R signaling has been recently shown to promote colitis in a murine model, suggesting that the balance between H_2R and H_4R may be critical in controlling mucosal inflammatory responses.[23,24] Recently, the expression of H_4R by peripheral blood monocytes has been questioned, with high interindividual variability of monocyte-specific H_4R gene expression being reported.[25] However, expression of this receptor can be influenced by pro-inflammatory stimuli, and increased H_4R staining of monocytes from patients with IBD may be due to ongoing inflammatory responses. Our flow cytometry staining of B cells suggest that B cells from healthy volunteers do not express the H_4R (less than 0.3% positive B cells), which agrees with previously published B-cell data sets.[26] However, a statistically significant, but numerically minor, increase in H_4R staining was observed for B cells from patients with ulcerative colitis. This suggests that a relatively rare subpopulation of B cells may upregulate H_4R in patients with ulcerative colitis. In addition to altered receptor expression, differences in the intracellular signaling mechanisms associated with the H_2R pathway may be important. H_2R modulation of TLR activation is 3′,5′-cyclic adenosine monophosphate dependent, with downstream effects on NF-κB activation and cytokine secretion being mediated by exchange protein directly activated by cyclic adenosine monophosphate (Epac).[10] Epac has also been shown to be responsible for other immune regulatory activities such as mediating dendritic cell migration to T_{REG}-derived adenosine, rendering them less stimulatory.[27] It is currently unknown if Epac expression or activity is modulated in patients with IBD. Regardless of the mechanism, it is clear that PBMCs from patients with IBD respond differently to histamine compared with cells from healthy volunteers.

Histamine does not suppress all TLR-induced cytokines but there clearly is specificity for specific TLR type–cytokine combinations, which was previously described by us for dendritic cells.[10] As TNF-α and IFN-γ secretion were the most consistent cytokines suppressed for the majority of TLRs, we evaluated gene expression of these cytokines and histamine receptors in mucosal tissue. Expression of proinflammatory cytokines, such as TNF-α and IFN-γ, was increased in the inflamed compared with noninflamed mucosa from the same individuals. Surprisingly, expression of TNF-α positively correlated with H_2R expression, whereas IFN-γ expression positively correlated with H_4R expression in ulcerative colitis patients. Such a close association suggests that one of these factors may influence the expression of the other or a separate factor induces all these genes. One might expect that increased expression of H_2R would correlate with decreased, rather than increased, TNF-α gene expression. Similarly, other regulatory factors, such as IL-10, have also been previously seen to be increased in inflamed tissue, suggesting that the inflamed mucosal microenvironment drives expression of regulatory factors, which unfortunately are not sufficient to fully reverse the inflammatory response. However, loss of IL-10 or H_2R exacerbates colitis in experimental models, which suggests that these molecules do exert some anti-inflammatory effects, albeit not potent enough to reverse disease.[28] Another surprising finding is that these cytokine–histamine receptor associations were only seen in ulcerative colitis patients but not in patients with Crohn's disease, suggesting that the ulcerative colitis inflammatory process may associate to a greater extent with dysregulated histamine responses compared with the mucosal microenvironment of patients with Crohn's disease. The specific cytokines that directly influence histamine receptor expression are currently unknown, but future studies should examine the differential influence of T_H1, T_H2, and T_H17 cytokines on histamine receptor expression within the mucosa. One limitation of our studies on mucosal biopsies is that gene expression analysis of the entire biopsy does not allow us to identify which cell types are differentially expressing histamine receptors within the inflamed mucosa. One possibility for increased histamine receptor expression is that certain cell types, potentially including epithelial cells, dendritic cells, macrophages, innate lymphoid cells, invariant natural killer T cells, T or B lymphocytes, may upregulate histamine receptor expression. However, it is also possible that recruitment of histamine receptor expressing cells to the inflamed tissue could alter gene expression levels. Interestingly, *H_2R* gene expression was significantly increased in the inflamed colon of mice that received lymphocytes from H_2R-deficient donors, suggesting that cells other than lymphocytes express the *H_2R* in this model. Regardless, the high level of histamine receptor expression within inflamed mucosa suggests that these cells are susceptible to the immunoregulatory influence of histamine.

Previously, other reports have described that histamine levels are increased within the mucosa of patients with IBD, which potentially could be due to an imbalance in histamine synthesis or metabolism. *HNMT* gene expression was reported to be reduced in inflamed mucosa, and DAO polymorphisms have been associated with an increased risk of IBD.[29,30] We did not observe any differences in gene expression for *HDC*, *HNMT*, or *DAO* in inflamed compared with noninflamed mucosa; however, there was a decrease in *DAO* gene expression within peripheral blood of patients with

IBD, which may contribute to elevated levels of histamine previously reported in these patients. Although histamine levels are important, the binding of histamine to different histamine receptors is perhaps more relevant and decisive. Recently, the use of H_2R antagonists, but not proton pump inhibitors, significantly increased the risk of hospitalization or surgery in Crohn's disease patients.[31] In addition, we describe that transfer of T cells, which lack H_2R, or inhibition of H_2R using a specific antagonist accelerates weight loss and increases disease severity in a mouse colitis model. These results suggest that histamine signaling through the H_2R may have protective effects within the mucosa.

In addition to IBD, inappropriate TLR signaling has been associated with multiple inflammatory and infectious diseases, particularly those of mucosal tissues.[32–34] There is increasing interest in the role that histamine plays in many of these pathologies.[35] Taken together, it is clear that histamine and histamine receptors are intimately connected to the fine-tuning of innate immune responses to microbes and appropriate TLR signaling. Indeed, certain microbes can secrete histamine within the gut and may directly influence gastrointestinal inflammation through H_2R.[13,36] The deliberate activation of H_2R, or its associated signaling pathways, is a novel therapeutic target for inflammatory diseases. However, the microenvironmental or genetic polymorphisms that influence histamine receptor expression and/or activity remain to be determined.

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